Irradiation Disinfestation of Apple Maggot (Diptera: Tephritidae) in Hypoxic and Low-Temperature Storage

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ABSTRACT Apple maggot, *Rhagoletis pomonella* (Walsh), is a quarantine pest of apples, *Malus domestica* Borkhausen, and pears, *Pyrus communis* L., shipped from much of the United States and Canada. As such, these fruits shipped from infested areas to uninfested areas must undergo a quarantine disinfestation treatment. The objective of this research was to develop irradiation quarantine treatments against apple maggot considering that fruit hosts may be stored under hypoxic or cold conditions when they are irradiated. Hypoxia increased from 30.5 to 35.7 Gy (17%) the estimated dose to achieve 99% prevention of the full pupal stage from irradiated third instars in apples compared with ambient atmospheres. However, 50 Gy completely prevented the full pupa in 22,360 and 15,530 third instars, respectively, irradiated in apples in ambient and hypoxic atmospheres. There was no difference in development to the full pupal stage in apple maggot third instars held at 1 or 24°C when irradiated with 20 Gy. Because the maximum dose measured when 50 Gy was sought was 57 Gy, the latter should be the dose recommended for quarantine disinfestation of host fruits of the apple maggot. Apples and pears tolerate much higher doses.

KEY WORDS *Rhagoletis pomonella*, quarantine treatment, controlled atmosphere, cold storage, phytosanitary treatment

APPLE MAGGOT, *Rhagoletis pomonella* (Walsh), is a pest of apples, *Malus domestica* Borkhausen, and pears, *Pyrus communis* L., and native to temperate North America east of the Rocky Mountains. A population in central Mexico may be a distinct species (Smith and Bush 1997). Apple maggot has become established in parts of western North America, but nowhere else. If apple maggot host fruit from infested areas is to be shipped to a noninfested area where the fly could become established, the fruit must undergo a phytosanitary procedure that will ensure that no viable insects accompany it. Methyl bromide fumigation is the principal apple maggot disinfestation treatment. The fumigant has been implicated as a significant stratospheric ozone-depleting substance, and its use is being restricted worldwide by the Montreal Protocol. Although postharvest quarantine uses of methyl bromide have been exempted, there is no certainty that it will not be lost as a quarantine treatment or at least be more scarce and expensive. The price of the fumigant has more than quadrupled in recent years (Hallman 2003). Alternative phytosanitary treatments are needed. Cold storage for 40 d at 0°C is approved by regulatory agencies and tolerated by apples, but quicker alternatives are sought to better respond to markets.

Irradiation quarantine treatments have been used continuously in the United States since 1995 (Hallman 2001). Commodities are exposed to electron beams, X rays converted from an electron beam generator, or gamma rays from the isotopes Cobalt 60 or Cesium 137. The mode of action of irradiation involves breaking chemical bonds, preventing normal development or reproduction of the organism. It is a random process so large molecules, such as DNA, are the most likely to be rendered inoperable. There is no residue, and irradiation does not make food harmful to consumers (WHO 1994). Efficacy, commodity tolerance, consumer acceptance, and cost of irradiation have been favorable for quarantine applications, and its expanded use in this regard is probable.

A quarantine treatment must be virtually 100% effective. Irradiation is unique among applied quarantine treatments in that the objective is not acute mortality but prevention of development or reproduction. Achieving 100% acute mortality requires doses of irradiation not tolerated by fresh commodities and is not necessary to prevent the establishment of a pest. Apples and pears tolerate between 300 and 900 Gy of radiation, depending on the cultivar (Drake et al. 1999). Efficacy of irradiation research with tephritids usually is measured by failure of adult emergence from the puparium (Hallman and Loaharanu 2002). That works well with tropical tephritids that have a 2-wk puparial period, but temperate tephritids diapause as fully developed pupae and adults may not emerge for up to 3 yr. Hallman and Thomas (1999) solved this problem by using failure to form the full pupa as the

measure of efficacy for two species of *Rhagoletis.* This results in a somewhat higher dose than that possible when prevention of adult emergence is the criterion, but doses for tephritids are relatively low regardless. Hallman and Thomas (1999) estimated that doses between 29 and 152 Gy would achieve quarantine security against apple maggot. Research to narrow this range and confirm a dose with sufficient numbers of insects tested is needed to be able to use irradiation as a commercial quarantine treatment against apple maggot.

Hosts of apple maggot are often stored under hypoxic conditions to prolong shelf life (Blanpied 1990). Hypoxia is known to reduce the effects of radiation on organisms because less oxidative radicals, responsible for some of the radiation injury, are produced (von Sonntag 1987). Hypoxia increased oriental fruit moth, *Grapholita molesta* (Busck), radiotolerance at least two-fold (G.J.H., unpublished data). Hallman and Worley (1999) found that Mexican fruit fly, *Anastrepha ludens* (Loew), third instars irradiated in vitro in a nitrogen-purged atmosphere were twice as radiotolerant as those irradiated in air.

Hosts of apple maggot are usually stored at temperatures near 0°C, at which freezing of the fruits or insects does not occur. Although freezing lessens radioinduced inactivation of microorganisms, because in the solid state the reactive intermediates of water radiolysis are not able to interact with the cells as freely, differences in temperature above freezing do not seem to affect the efficacy of microorganism inactivation (Stewart 2001). Macfarlane (1966) found that failure of irradiated Queensland fruit fly, *Bactrocera tryoni* (Froggatt), to pupariate increased with increasing temperature between 4.5 and 36°C. Adult emergence occurred only at the lowest temperature. Pendelbury (1966) found no difference in sterility of granary weevil, *Sitophilus granarius* (L.), held at 15 or 30°C before, during, or after irradiation.

The objectives of this research were to determine the effects of hypoxic and cold storage separately on the efficacy of irradiation disinfestation against apple maggot and to treat enough insects to confirm a dose that could be used as a commercial phytosanitary procedure.

Materials and Methods

Apple Maggot. Apple maggots were collected as larvae in apples in the field near Biglerville, PA, and reared on organically grown 'Delicious' apples from Washington State. About a dozen apples each were placed in plastic-screened cages (28 cm^3) at 24°C with ≈100 apple maggot adults (50:50 sex ratio) for 3–4 d. Apples were incubated for ≈2 wk until late third instars, the most radiotolerant stage found in fruit (Hallman and Loaharanu 2002), were formed. Voucher specimens were deposited in the collection at USDA–ARS–CQFIRU Weslaco.

Irradiation System. A ^{137}Cs source (Husman model 521A, Isomedix, Inc., Whippany, NJ) delivering a centerline dose rate of ≈40 $Gy \cdot min^{-1}$, was used in this research. Reference standard dosimetry was done in 1996 with the Fricke system. Routine dosimetry was done with film (Gafchromic MD-55, ISP Technologies, Inc., Wayne, NJ) and read with a spectrophotometer (Milton Roy Spectronic 401, Ivyland, PA) at 510 nm. Routine dosimeters were placed about every eight cylinders in the center and the outside center of the load. These locations received the minimum and maximum absorbed doses, respectively, as determined by previous tests.

Radiotolerance in Ambient and Hypoxic Atmospheres. 'Delicious' Apples (≈6.5 cm in diameter) infested with third instars of apple maggots, via the process described above, were placed in polyvinyl chloride cylinders (37.5 cm inside length, 10 cm i.d.) fitted on one end with a screw cap sealed with vacuum grease and on the other end with two brass compression hose fittings (25 mm in length, 4 mm i.d.). Five apples were placed inside the cylinder, and the atmosphere was periodically purged through the hose fittings with nitrogen at a pressure of ≈3 kPa for 2 min at 20, 16, and 2 h before irradiation. The hose fittings were then sealed with rubber septa. The cylinders were irradiated with 30, 40, or 50 Gy. Approximately 1.5 h after irradiation, the cylinders were opened to return the larvae to ambient atmosphere. Checks on the effect of hypoxia alone for the 22 h that the apples were kept in hypoxic conditions consisted of the same procedure without irradiation. Another control consisted of larvae in apples that made the trip to the irradiator in unpurged cylinders that were not irradiated.

After irradiation, apples were removed from the cylinders and placed in plastic bins (30 by 23 by 12 cm) at 24°C to permit continued development of apple maggot. Larvae were collected as they emerged from apples and maintained in moist vermiculite to await pupariation and adult emergence. Once the apples began to decompose, they were opened and the remaining larvae were collected. Six weeks after pupariation, puparia from which adults had not emerged were opened and the contents separated into the following categories: dead adults, live (diapausing) pupae, dead pupae, fungal mass with no discernible insect stage, any dead prepupal stage, or largely hollow puparium. The hollow puparium was probably an insect that did not survive long after pupariation, hence, did not have much chitinous structure. The process was repeated until a few thousand third instars had been treated at each dose–atmosphere combination. When it became apparent that 50 Gy would prevent pupation, >15,000 larvae were irradiated at that dose under both atmospheres to confirm efficacy with large numbers of insects. Data were analyzed with PROC PROBIT (SAS Institute 1988).

Radiotolerance at Low Temperature Storage. Eighty apples infested with third instars as before were separated into two groups, and one group was placed in a walk-in chamber at 1 ± 0.5°C ≈20 h before irradiation. The other group was left at ≈24°C. Approximately 1 h before irradiation, the apples in cold storage were removed from the chamber and placed in

coolers with ice for the trip to and from the irradiation facility (45 min each way). Half of each of the apple groups stored at 1 and 24°C was irradiated with 20 Gy, which took ≈1 min to deliver, before the 1°C group was returned to the coolers. Upon returning to the laboratory, all of the apples were removed from the coolers (after ≈22 h at 1°C) and placed in plastic bins as before. Larvae were collected and maintained in moist vermiculite, and resulting puparia were examined as described above. The test was replicated four times.

Dose–response data were analyzed with the probit procedure using the normal probability density function with $\log_{10}$ of dose (SAS Institute 1988).

Results and Discussion

The fate of third instars of apple maggots from irradiated apples is given in Table 1. The largest loss was in failure of third instars to pupariate; even in the controls one-third failed to pupariate. Pupariation failure rates of 50% are common for *Rhagoletis*, which are more difficult to rear than many tropical tephritids (Boller 1989). For irradiated larvae, rates of puparial failure were from 58 to 95%, indicating that radiation exacerbated that problem. Emerged adults and live, diapausing pupae were not found at doses >30 Gy. Development did not proceed to the pupal stage at >40 Gy. Preventing formation of the full pupa from apple maggot third instars in apples irradiated under ambient atmospheres fit the probit model using the normal probability density function with the $\log_{10}$ of dose (n = 25,562; slope + SE = 9.67 + 1.95; effective dose $(ED)_{99}$ = 30.5 Gy; 95% CL = 29.6–31.5 Gy; χ^2 = 0.67). The ED_{99} was almost one-half of that found by Hallman and Thomas (1999), which was ED_{99} = 58.3 Gy; 95% CL 44.3–139 Gy. However, the data in that study did not fit the model used, which was the same model as in the current study.

Preventing formation of the full pupa from apple maggot third instars in apples irradiated under hypoxic conditions also fit the probit model by using the normal probability density function with the $\log_{10}$ of dose (n = 17,093; slope + SE = 17.5 + 1.66; ED_{99} = 35.7 Gy; 95% CL = 34.8–37.2 Gy; χ^2 = 0.93).

Mean ± SEM prevention of the full pupa from apple maggot third instars in apples irradiated at 1 or 24°C was almost identical (93.6 ± 2.5 and 93.8 ± 1.8%, respectively). Because these results were so close, there was no need for an analysis of variance (ANOVA), and it was concluded that temperature in the range studied does not affect efficacy of irradiation against apple maggot. Ninety-three and 85% of third instars that reached the full pupal stage when irradiated at 1 or 24°C, respectively, died as adults inside puparia; the rest were pupae in diapause.

Although hypoxia increased apple maggot radiotolerance by an estimated 17%, as measured by prevention of the pupa from irradiated third instars, under ambient or hypoxic atmospheric conditions, a target, absorbed dose of 50 Gy applied to third instars of apple maggot prevented pupation. The number of third instars irradiated at 50 Gy with none reaching the full pupa was 22,360 and 15,530, respectively, for ambient and hypoxic atmospheres. Hallman and Worley (1999) found that Mexican fruit fly third instars irradiated in a nitrogen-purged atmosphere were twice as radiotolerant as those irradiated in air. However, those irradiated inside grapefruits *Citrus paradisi* Macf., were twice again as radiotolerant as those irradiated outside of fruit but in a nitrogen-purged atmosphere. Although the atmosphere inside of grapefruits is hypoxic, it is not severely deficient in oxygen, containing about one-half the ambient oxygen level (Hallman et al. 1994). The authors concluded that other factors besides hypoxia contribute to making Mexican fruit fly larvae inside grapefruits more radiotolerant than larvae outside of fruit, whether in ambient or hypoxic atmospheres.

Because the maximum dose measured when 50 Gy was sought was 57 Gy, the latter should be the dose recommended for quarantine disinfestation purposes. This dose is higher than the absolute minimum that would achieve quarantine security against apple maggot in apples, because the objective, prevention of pupation, requires a higher dose than the usual objective of irradiation quarantine treatments against tephritids, which is prevention of adult emergence.

Table 1. Fate of third instars of apple maggot irradiated in apples in ambient and hypoxic atmospheres

Target dose (Gy)	n	Oxygen level	Failure to pupariate (%)	Pupariated, but not pupated			Pupae		Adults		
				Died as larvae (%)	Hollow (%)	Fungi (%)	Dead (%)	Alive (%)	Dead in puparium (%)	Partially emerged (%)	Fully emerged (%)
0 (control)	4,275	Ambient	34.2	5.9	0.07	1.3	0	6.7	7.7	0	44.1
0 (control)	2,902	Hypoxic	32.2	9.4	0.1	1.8	0	8.5	9.1	0	38.9
30	3,224	Ambient	84.0	10.5	2.0	2.4	0	0.03	0.84	0.06	0.28
30	1,491	Hypoxic	57.9	18.7	2.2	5.1	0.54	2.2	12.5	0.34	0.47
40	2,847	Ambient	95.2	3.6	0.03	1.1	0	0	0.04	0	0
40	2,698	Hypoxic	73.3	20.0	1.1	5.5	0.04	0	0.04	0	0
50	19,491	Ambient	87.5	11.2	0	1.4	0	0	0	0	0
50	12,904	Hypoxic	78.4	19.3	0.01	2.3			0	0	0

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